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Cannabimimetic Lipid Amides as Useful Medications

Background of the Inventi n

Classical cannabinoids such as the marijuana derived cannabinoid Δ^9-tetrahydrocannabinol, (Δ^9-THC) produce their pharmacological effects through interaction with specific cannabinoid receptors in the body. So far, two cannabinoid receptors have been characterized: CB1 found in the mammalian brain and peripheral tissues and CB2 found only in the peripheral tissues. Compounds which stimulate those receptors have been shown to induce analgesia and sedation, to cause mood elevation including euphoria and dream states, to control nausea and appetite and to lower intraocular pressure. Cannabinoids have also been shown to suppress the immune system and affect the reproductive system. Thus, compounds which stimulate the CB1 and CB2 receptors, directly or indirectly, are potentially useful as oral and topical contraceptive preparations, in treating glaucoma, preventing tissue rejection in organ transplant patients, controlling nausea in patients undergoing chemotherapy, controlling pain and enhancing the appetite in individuals with AIDS Wasting Syndrome.

In addition to acting at the cannabinoid receptors, cannabinoids such as Δ^9-THC also affect cellular membranes, thereby producing undesirable side effects such as drowsiness, impairment of monoamine oxidase function and impairment of non-receptor mediated brain function. The addictive and psychotropic properties of cannabinoids also limit their therapeutic value.

Arachidonylethanolamide (anandamide) is an endogenous lipid that binds to and activates the CB1 cannabinoid receptor with approximately equal affinity to that of Δ^9-THC. Anandamide also exhibits biochemical and pharmacological properties similar to that of Δ^9-THC, albeit with a longer onset time and shorter duration of action. The exact physiological role of anandamide, a cannabinoid agonist, is still not clearly understood. It is known that an enzyme called "anandamide

amidase" hydrolyzes anandamide. It is presumed that the magnitude of action and relatively short duration of action of anandamide is due to a rapid inactivation process consisting of carrier-mediated transport into cells followed by intra-cellular hydrolysis by anandamide amidase.

There is considerable interest in developing analogs of anandamide possessing high CB1 receptor affinity and/or metabolic stability. Such analogs may offer a rational therapeutic approach to a variety of disease states, including pain, psychomotor disorders, and multiple sclerosis, in which elevation of anandamide analog levels may bring about a more favorable response with fewer side effects and greater metabolic stability than direct activation of CB1 receptors by anandamide.

Summary of the Invention

It has now been found that certain novel analogs of anandamide possess improved CB1 receptor affinity and selectivity and/or greater metabolic stability than anandamide. The term "metabolic stability" as used herein refers to the resistance to hydrolysis of the subject anandamide analog by anandamide amidase.

The analogs were prepared by structural modification of anandamide. The modifications were primarily made in the ethanolamido head group and comprised the substitution or addition of alkyl, substituted alkyl, alkenyl and alkynyl groups. Additionally, a number of retro-anandamides, in which the positions of the NH and CO groups are reversed, were prepared. The retro-anandamides comprised the substitution or addition of alkylacetoxy groups. The analogs prepared are summarized in Table 1.

Based on the results of the prepared analogs, it is believed that a number of additional analogs of anandamide and retro-anandamide would provide similar physiological results. These additional analogs

comprise the headgroup substitution or addition of alkyl, substituted alkyl, alkenyl, alkynyl and alkylacetoxy groups, as well as cycloalkyl, polycyclic and heterocyclic groups. Further, structural modification may be made to the tail of the anandamide and retro-anandamide analogs, comprising substitution or addition of alkyl, substituted alkyl, O-alkyl, aryl, alkylaryl, O-alkylaryl, cyclic and heterocyclic groups. These analogs are represented in Table 2.

The improved receptor affinity and selectivity and/or metabolic stability create therapeutic uses for the novel analogs. For example, the compounds of the present invention can be effective in the relief of the pain caused by cancer and the nausea resulting from cancer chemotherapy as well as for the relief of peripheral pain. In addition, the compounds disclosed herein may be immunosuppressive and can therefore be used to prevent organ rejection in an individual undergoing an organ transplant. Because the compounds of the present invention enhance the appetite of an individual, they can be used to treat patients with AIDS Wasting Syndrome, who are often suffering from malnourishment as a result of appetite loss. The compounds could also be used to treat psychomotor disorders, multiple sclerosis, peripheral hypertension and as oral and topical contraceptives.

Description of Some Preferred Embodiments

The known physiological activity of cannabinoids, such as Δ^9-THC, and endogenous lipids, such as anandamide, indicate that other novel lipid materials may also interact with the specific cannabinoid receptors. Further, the novel lipid materials can be more resistant to hydrolysis by anandamide amidase than is anandamide, providing a greater magnitude of action and longer duration of action than anandamide. Additionally, the novel lipid materials can have high r affinities for specific cannabinoid receptors than anandamide. Specific

analogs of anandamide and retro-anandamide were prepared and tested according to the procedures and protocols discussed below.

Typical procedures for synthesizing the novel analogs of anandamide are as follows:

3(1'R)-1'-Methyl-2'-hydroxyethly)-4,5-diphenyloxazolidin-2-one. A solution of 1.16 g(15.4 mmol) of R-alaninol in 12 mL of anhydrous dimethylformamide was cooled in an ice-bath. 4,5-Diphenyl-1,3-dioxol-2-one 3.67 g (15.4 mmol) was added in one portion and the resulting solution was stirred at room temperature for 1.5 hours. At the end of time period, the reaction mixture was diluted with 50 mL of water and extracted with ethyl acetate (75 mL, 50 mL). Combined organic extracts were dried ($MgSO_4$) and evaporated to afford a colorless oil to which 7 mL of anhydrous trifluoroacetic acid was added and the resulting solution was allowed to stand at room temperature for 2 hours. Methylene chloride was added to the reaction mixture which was found to be a mixture of the title product and the corresponding trifluoroacetate ester. The solution was washed with water, 10% sodium bicarbonate, water and dried ($MgSO_4$). Rotary evaporation of the solvent gave an oil which was dissolved in 15 mL of 95% methanol, 1.5 g of potassium carbonate was added and the heterogenous mixture was stirred for 5 min at room temperature. After dilution with water, the mixture was extracted with methylene chloride, combined organic extracts were dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (80% ethyl-petroleum ether) to afford a white solid (4.0 g, 92%); m.p. 126-128 °C, Optical rotation +23.6 (16.4, $CHCl_3$). ^{13}C NMR ($CDCl_3$), δ 130.9, 130.3, 129.6, 128.4, 127.7, 124.3, 64.0, 52.4, 14.7.

3((1'R)-1' Methyl-2'-fluor thyl)-4,5 diphenyloxazolidin-2-one. A solution of 1.08 mL (8.14 mmol) of diethylaminosulfur trifluoride

(DAST) in 40 mL of anhydrous methylene chloride was cooled to -78 °C and a solution of 2.0 g (6.78 mmol) of "ox" protected R-alaninol was added dropwise over 5-10 min. The cooling bath was removed and the reaction mixture was stirred at room temperature for 5 hours. Reaction was quenched by addition of 5 mL of water, organic layer was separated, dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (50% ethyl ether-petroleum ether) to afford 0.9 g (74%) of white solid. m.p. 127-129 °C, Optical rotation -6.56° (15.3, $CHC1_3$), ^{13}C NMR ($CDCl_3$) δ 131.1, 130.4, 129.7, 128.5, 127.7, 124.4, 82.2 (d, J CF=172.7 Hz), 50.2 (d, J_{CF}=19.8 Hz, 13.4 (d, J_{CF}=6.5 Hz).

Analog 1, N-(2-Fluoro-1R-(methylethyl) arachidonylamide). 3(1'R)-l'-Methyl-2'-fluoroethyl) 4,5-diphenyloxazolidim 2-one (100 mg, 0.34 mmol) was dissolved in 14 mL of absolute ethanol. Palladium on charcoal (10%) 57 mg was added and the solution was hydrogenated at 45 psi for 30 hours. After acidification with conc. HCl, solution was filtered through celite. The filtrate was concentrated to afford the HCl salt of (2R)-1-fluoro-2-propylamine, which was coupled with arachidonic acid chloride as described below.

In another flask, a solution of 100 mg (0.33 mmol) of arachidonic acid and 0.06 mL of dry dimethylformamide in 2 mL of dry methylene chloride was cooled in an ice-bath. Then a 2 M solution of oxalyl chloride in methylene chloride (0.33 mL, 2 equiv) was added dropwise. Reaction mixture was stirred at ice-bath temperature under argon for 1 hour. The above (2R)-1-fluoro-2-propylamine as a solution in 0.5 mL of pyridine was added and the reaction mixture was stirred at room temperature for 30 min. The solution was transferred to a separatory funnel and washed with 10% hydrochloric acid, water, and dried. After rotary evaporation, the residue was purified by column chromatography

on silica gel (50% ethyl ether-petroleum ether) as eluent to afford colorless oil: [α] +10.82 (4.62, $CHC1_3$).

Analog 2, N-(2-Fluoro-IS-(methylethyl) arachidonylamide, was prepared using the same procedure from N-t-BOC-S-alaninol.

Tosylate of (N-t-BOC-R-Alaninol). A solution of N-t-BOC-R-alaninol (1.4 g, 7.99 mmol) and 1.95 mL (24 mmol) of dry pyridine in 7 mL of dry chloroform was cooled in an ice-bath and a solution of p-toluenesulfonylchloride (2.67 g, 16 mmol) in 3 mL of dry chloroform was added portionwise over a period of 3 hours. The reaction mixture was stirred at ice-bath temperature for 2 hours and then transferred to a separatory funnel with more chloroform and washed successively with 10% hydrochloric acid, 10% sodium bicarbonate and water and dried ($MgSO_4$). Solvent was removed and the residue was purified by column chromatography on silica gel to afford 2.20 g (84%) of viscous colorless oil.

3-Azido-2-(N-t-BOC-amino) propane. Sodium azide (3.62 g, 56 mmol) was added to a solution of the above tosylate (1.0 g, 3.04 mmol) and the heterogenous mixture was heated in an oil-bath at 60-70 °C, with stirring, for 2 hours [CAUTION: Use a safety shield]. The reaction mixture was cooled to room temperature, poured into water and extracted with diethyl ether. Combined ether extracts were dried ($MgSO_4$) and ether evaporated. The residue was purified by column chromatography to afford 354 mg (58%) of a colorless oil.

Analog 4, (R)-(+-)-Arachidonyl-1'-azido-2'-propylamide. The above azide was cooled in an ice-bath and 3 mL of dry trifluoroacetic acid was added. The flask was stoppered and the light yellow solution was stirred at room temperature for 2 hours. Most of the trifluoroacetic acid was removed in vacuo. In another flask, arachidonic acid chloride

was prepared from 100 mg (0.33 mmol) of arachidonic acid as described in our previous publication. A solution of 1.5 mL of anhydrous pyridine was added at 0 °C. The reaction mixture was stirred at room temperature for 30 min and then transferred into a separatory funnel using additional dichloromethane. The solution was washed successively with 10% hydrochloric acid, water, dried ($MgSO_4$) and solvents removed. The residue was purified by column chromatography (30-40% diethyl ether-petroleum ether) to give (82%) of analog 4.

Analog 5, N-(2-Chloroethyl)arachidonylamide. A solution of arachidonic acid (50 mg, 0.165 mmol) and 0.03 mL of anhydrous DMF in 1 mL of dry dichloromethane was cooled in an ice bath under argon and 0.17 mL of a 2 M solution of oxalyl chloride (0.34 mmol) in dichloromethane was added dropwise. Reaction mixture was stirred further at ice bath temperature for 1 hour. A solution of 65 mg (0.50 mmol, 3 equiv) of 2-chloroethylamine hydrochloride in 0.5 mL of dry pyridine was added, the cooling bath was removed, and the solution was stirred at room temperature for 30 min. The mixture was transferred to a separatory funnel, washed with 10% aqueous hydrochloric acid and water, and dried ($MgSO_4$). After rotary evaporation of solvents, the residue was chromatographed on silica gel (60% ethyl ether-petroleum ether) to afford 54 mg (90%) of the pure title compound as an oil: R_f (70% ethyl ether-petroleum ether) 0.35; ^1H NMR (270 MH_Z, $CDCl_3$) 5.80 (br s, 1H), 5.36 (m, 8H), 3.61 (m, 4H), 2.80 (m, 6H), 2.21 (t, J = 7.89 Hz, 2H), 2.10 (m, 4H), 1.72 (m, 2H), 1.29 (m, 6H), 0.88 (t, J = 6.79 Hz, 3H). Anal.($C_{22}H_{36}ClNO$) C, H, N.

Analog 6, *N*-(3-Chloropropyl)arachidonylamide. The title amide was prepared from 50 mg (0.165 mol) of arachidonic acid as described

for analog 5: yield 55 mg (88%); R_f (70% ethyl ether-petroleum ether) 0.35; ^{1}H NMR (270 MH_Z, $CDCl_3$) 5.60 (br s, 1H), 5.36 (m, 8H), 3.57 (t, J = 6.40 Hz, 2H), 3.40 (q, J = 6.45 Hz, 2h), 2.80 (m, 6H), 2.18 (t, J = 7.91 Hz, 2H), 2.01 (m, 6H), 1.70 (m, 2H), 1.29 (m, 6H), 0.88 (t, J = 6.89 Hz, 3H). Anal. ($C_{22}H_{38}ClNO$) C, H, N.

Analog 7, N-Allylarachidonylamide. Arachidonic acid chloride was prepared from 50 mg (0.165 mmol) of arachidonic acid as described under analog 5 and treated with 0.061 mL (0.83 mmol, 5 equiv) of allylamine. Similar work up followed by column chromatographic purification gave 48 mg (85%) of the pure title compound as an oil: R_f (40% ethyl ether-petroleum ether) 0.18; ^{1}H NMR (270 MH_Z, $CDCl_3$) 5.83 (m, 1H), 5.36 (m, 9H), 5.14 (m, 2H), 3.87 (m, 2H), 2.80 (m, 6H), 2.19 (t, J = 7.93 Hz, 2H), 2.10 (m, 4H), 1.72 (m, 2H), 1.29 (m, 6H), 0.88 (t, J = 6.74 Hz, 3H). Anal. ($C_{23}H_{37}NO$) C, H, N.

Analog 8, *N*-Propargylarachidonylamide. Arachidonic acid chloride was prepared from 50 mg (0.165 mmol) of arachidonic acid as described above for analog 5 and treated with 0.057 mL (0.83 mmol, 5 equiv) of propargylamine. Similar work up followed by column chromatographic purification gave 47.8 mg (85%) of the pure title compound as an oil: R_f (70% ethyl ether-petroleum ether) 0.30; H^1 NMR (270 MHZ, $CDCl_3$) 5.57 (br s, 1H, 5.36 (m, 8H), 4.04 (m, 2H), 2.80 (m, 6H), 2.22-2.00 (m, 7H), 2.01 (m, 6H), 1.72 (m, 2H), 1.29 (m, 6H), 0.88 (t, J=6.73 Hz, 3H). Anal. ($C_{23}H_{35}NO$) C, H, N.

Analog 9, *N*-(2,2,2-Trifluroethyl) arachidonylamide. Arachidonic acid chloride was prepared from 50 mg (0.165 mmol) of arachidonic acid as described under analog 5 and reacted with a solution of 111.8 mg (0.825 mmol, 5 equiv) of 2,2,2-trifluoroethylamine hydrochloride in

0.5 mL of pyridine. Reaction mixture was stirred at room temperature for 30 min and worked up in a similar manner to give 47.4 mg (75%) of the title amide: R_f (35% ethyl ether-petroleum ether) 0.20; ^1H NMR (270) MHZ, $CDCl_3$) 5.60 (br s, 1H), 5.36 (m, 8H), 3.91 (m, 2H), 2.80 (m, 6H), 2.24 (t, J = 7.39 Hz, 2H), 2.08 (m, 6H), 2.01 (m, 6H), 1.74 (m, 2H), 1.29 (m, 6H), 0.88 (t, J = 6.56 Hz, 3H). Anal. ($C_{22}H_{34}F_3NO$) C, H, N.

Arachidonyl alcohol: To a magnetically stirred solution of 0.5 ml (0.5 mmol) of $LiAlH_4$ in Et_2O, 100 mg (0.314 mmol) of arachidonic acid methyl ester in 2 mL of Et_2O was added dropwise at 0°C. The reaction mixture was stirred for 1 h and then quenched by addition of 1 mL of EtOAC. 2 mL of saturated NH_4Cl solution was added and the organic layer was separated, dried with $MgSO_4$, filtered and evaporated. Chromatography on silica gel (eluents: CH_2Cl_2/petroleum ether up to 70% CH_2Cl_2), evaporation, followed by membrane filtration of a CH_2Cl_2 solution of the product gave 99.3 mg (0.292 mmol, 93% yield) of arachidonyl alcohol as a colorless oil: TLC ($CHCl_3$) R_f 0.28; ^1H NMR (200 MHZ, $CDCl_3$) δ 5.37 (m, 8 H), 3.61 (t, 2 H, J = 6 Hz), 2.79 (m, 6H), 2.08 (m, 4 H), 1.66 - 1.17 (m, 8 H), 0.92 (t, 3 H, J = 7 Hz); Anal. C, H.

Arachidonylamine. To a magnetically stirred solution of 50 mg (0.17 mmol) of arachidonyl alcohol in 1 mL of pyridine was added 29.2 mg (0.225 mmol) of mesyl chloride at 0 °C. After stirring for 5 hours, the reaction mixture was poured into 2 mL of cold water and extracted with diethyl ether (2 x 4 mL). The combined ether extracts were washed with 1 N sulfuric acid and saturated sodium bicarbonate solution and evaporated in vacuo. The crude mesylate was dissolved in 2 mL of anhydrous DMF, and then a solution of 6.5 mg (0.85 mmol) of sodium azide in 4 mL of anhydrous DMF was added at room

temperature. The reaction mixture was heated to 90 °C for 24 hours behind a safety shield. The mixture was cooled to room temperature, inorganic material was filtered off, and the filtrate was poured into 1 mL of cold water. Extraction with diethyl ether (2 x 6 mL), drying ($MgSO_4$), and evaporation gave an oily residue which was chromatographed on silica gel (petroleum ether) to afford 39 mg (73%) of arachidonyl azide as a colorless oil: ^{1}H NMR (200 MHZ, $CDCl_3$) δ 5.38 (m, 8H), 3.27 (t, J = 6 Hz, 2H), 2.81 (m, 6H), 2.11-2.01 (m, 4H), 1.62 (m, 2H), 1.48-1.25 (m, 6H), 0.89 (t, J = 7 Hz, 3H).

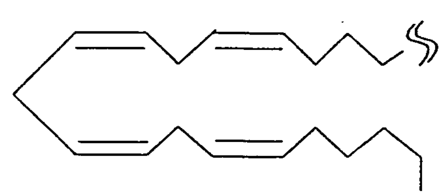
The crude azide was reduced to the title amine as follows: To a magnetically stirred solution of 132 mg (0.43 mmol) of arachidonyl azide in 3 mL of dry diethyl ether was added 4 mL of 1.0 M solution of lithium aluminum hydride (4.0 mmol) in THF dropwise at room temperature. The reaction mixture was refluxed for 3 hours and then quenched with wet diethyl ether. The white suspension was filtered, and the filtrate was evaporated to dryness. Chromatography on silica gel (10-50% MeOH in dichloromethane) gave 65 mg (51%) of arachidonylamine as a white solid: TLC (20% EtOAc-CH2Cl2) *Rf* 0.33; ^{1}H NMR (200 MHZ, $CDCl_3$) δ 5.38 (m, 8H), 2.82 (m, 6H), 2.70 (t, J = 6.6 Hz, 2H), 2.08 (m, 4H), 1.40 (m, 4H), 1.26 (m, 6H), 0.89 (t, J = 6.4 Hz, 3H). Anal. ($C_{20}H_{35}N$) C, H, N.

Analog 10, *N*-(3-Hydroxypropionyl)arachidonylamine. To a magnetically stirred solution of 48 mg (0.17 mmol) of arachidonylamine in 2 mL of anhydrous dichloromethane was added 58 μL of a 2.0 M solution of trimethylaluminum (0.17 mmol) in hexane at room temperature. The mixture was stirred for 20 min, and then 12.24 mg (0.17 mmol) of β-propiolactone was added. The reaction mixture was refluxed for 6 hours, quenched with 1 N HCl, and extracted with dichloromethane. The crude product was purified by column chromatography on silica gel (50-80% ethyl acetate in dichloromethane)

to afford 51 mg (83%) of the title compound as an oil: TLC (EtOAc) *R*f 0.26; ^{1}H NMR (200 MHZ, $CDCl_3$) δ 5.35 (m, 8H), 3.85 (q, *J* =5.4 Hz, 2H), 3.25 (q, *J* = 5.4 Hz, 2H), 2.84 (m, 6H), 2.66 (t, *J* = 6.8 Hz, 2H), 2.05 (m, 4H), 1.57 (m, 2H), 1.35 (m, 6H) 0.89 (t, *J* = 6.5 Hz, 3H). Anal. ($C_{23}H_{39}NO_2$) C, H, N.

Analog 11, *N*-(2-Acetoxyacetyl)arachidonylamine. To a magnetically stirred solution of 75 mg (0.26 mmol) of arachidonylamine in 2 mL of dry dichloromethane was added 40 μL (0.37 mmol) of acetoxyacetyl chloride at room temperature, and the mixture was stirred for 1 h. Excess acetoxyacetyl chloride was destroyed by adding 50 μL of water, solvents were evaporated, and the residue was chromatographed on silica gel (10-25% ethyl acetate in CH_2Cl_2) to afford 71 mg (70.2%) of the title amide as an oil: *R*f (EtOAc) 0.79; ^{1}H NMR (200 MHZ, $CDCl_3$) δ 5.37 (m, 8H), 4.55 (s, 3H), 3.32 (q, *J* = 7 Hz, 2H), 2.81 (m, 6H), 2.18 (s, 3H), 2.09 (m, 4H), 1.50-1.25, (m, 6H), 0.92 (t, *J* = 7 Hz, 3H). Anal. ($C_{24}H_{39}NO_3$) C, H, N.

Certain prepared anandamide analogs were assayed for cannabinoid receptor affinity following the protocol of Example 1. The test results are summarized in Table 1. As used herein, AA refers to a portion of the anandamide molecule having the structure:



EXAMPLE 1

It is known that the enzymatic action of anandamide amidase can be moderated or prevented in vitro by the inclusion of phenylmethanesulfonyl fluoride (PMSF). PMSF functions as a non-selective protease inhibitor. Thus the ligand binding determinations for the CB1 receptor were carried out in the presence and absence of PMSF, to obtain both CB1 receptor binding affinity and a relative measure of the analog's metabolic stability. The binding affinities (K_i) are expressed in nanomoles (nM).

For the CB1 receptor binding studies, membranes were prepared from rat forebrain membranes according to the procedure of P.R. Dodd et al, A Rapid Method for Preparing Synaptosomes: Comparison with Alternative Procedures, Brain Res., 107 - 118 (1981). The binding of the novel analogues to the CB1 cannabinoid receptor was assessed as described in W.A. Devane et al, Determination and Characterization of a Cannabinoid Receptor in a Rat Brain, Mol. Pharmacol., 34, 605 - 613 (1988) and A. Charalambous et al, 5'-azido Δ^8 - THC: A Novel Photoaffinity Label for the Cannabinoid Receptor, J. Med. Chem., 35, 3076 - 3079 (1992) with the following changes. The above articles are incorporated by reference herein.

Membranes, previously frozen at -80°C, were thawed on ice. To the stirred suspension was added three volumes of 25mM Tris-HCl buffer, 5 mM $MgCl_2$ and 1 mM EDTA, pH 7.4 (TME) containing 150 μM PMSF (made fresh in 2-propanol as a 100 mM stock). The suspension was incubated at 4°C, and after 15 min a second addition of PMSF stock brought the concentration to 300 μM PMSF; then the mixture was incubated for another 15 min. At the end of the second 15-min incubation, the membranes were pelleted and washed three times with TME to remove un-reacted PMSF.

The treated membranes were subsequently used in the binding assay described below. Approximately 30 μg of PMSF-treated membranes were incubated in silanized 96-well microtiter plate with TME containing 0.1% essentially fatty acid-free bovine serum albumin (BSA), 0.8 nM [^{3}H] CP-55,940, and various concentrations of anandamide analogues in a final volume of 200 °C for 1 hour. The samples were filtered using Packard Filtermate 196 and Whatman GF/C filterplates and washed with wash buffer (TME) containing 0.5% BSA. Radioactivity was detected using MicroScint 20 scintillation cocktail added directly to the dried filterplates, and the filterplates were counted using a Packard Instruments Top-Count. Nonspecific binding was assessed using 100 nM CP-55,940. Data collected from three independent experiments performed with duplicate determinations was normalized between 100% and 0% specific binding for [^{3}H] CP-55,940, determined using buffer and 100 nM CP-55,940. The normalized data was analyzed using a 4-parameter nonlinear logistic equation to yield IC_{50} values. Data from at least two independent experiments performed in duplicate was used to calculate IC_{50} values which were converted to K_i values using the using the assumptions of Cheng et al, <u>Relationship Between the Inhibition Constant (K_i) and the concentration of Inhibitor which causes 50% Inhibition (IC_{50}) of an Enzymatic Reaction</u>, Biochem. Pharmacol., 22, 3099-3102, (1973), which is incorporated by reference herein.

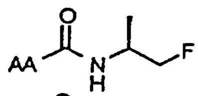
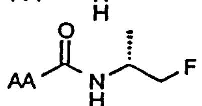
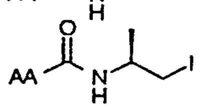
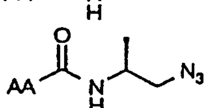
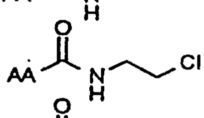
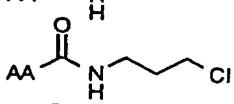
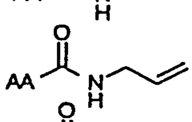
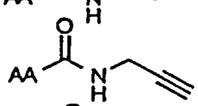
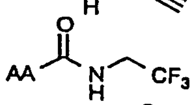
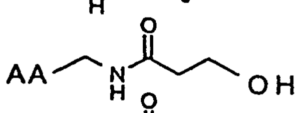
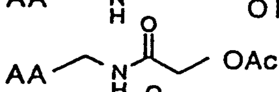
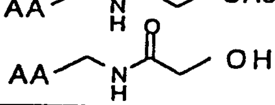
The CB1 ligand binding determinations in the absence of PMSF were performed in a similar manner to the above test, except without the use of PMSF.

For the CB2 receptor binding studies, membranes were prepared from frozen mouse spleen essentially according to the procedure of P.R. Dodd et al, <u>A Rapid Method for Preparing Synaptosomes: Comparison with Alternative Procedures</u>, Brain Res., 226, 107 - 118 (1981).

Silanized centrifuge tubes were used throughout to minimize receptor loss due to adsorption. The CB2 binding assay was conducted in the same manner as for the CB1 binding assay except the assays were conducted without PMSF. Since the CB2 receptor preparation has been shown to be devoid of anandamide amidase, the presence or absence of PMSF was not considered to be determinative. The binding affinities (K_i) are expressed in nanomoles (nM). The test results are summarized in Table 1.

TABLE 1.

Anandamide Analog Cannabinoid Receptor Site Binding Affinities

	Analog	K_i (CB1), nM		K_i (CB2), nM
		with PMSF	without PMSF	
1		1.18	10:1	785
2		0.91	56.3	1336
3		19.1	19.5	1394
4		12.7	25.6	1228
5		5.29	3400	195
6		25.6	387	193
7		9.91	2980	226
8		10.8	4900	290
9		36.2	2380	718
10		115	134	3540
11		421	419	>10000
12		20	23	-

With reference to Sonyuan LIN et al, Novel Analogues of Arachidonylethanolamide (Anandamide): Affinities for the CB1 and CB2 Cannabinoid Receptors and Metabolic Stability, Journal of Medicinal Chemistry, Vol 41, No 27, 5353 - 5361, (1998), which article is incorporated by reference herein, anandamide has been found to have a CB1 K_i of 61.0 nM (with PMSF); a CB1 K_i of 5810 nM (without PMSF); and a CB2 K_i of 1930 nM. As can be seen from Table 1, virtually all of the analogues have higher affinities to the CB1 and CB2 receptor sites than does anandamide. Further, most of the analogues exhibit much smaller differences for the CB1 affinities with and without PMSF, indicating greater metabolic stability than anandamide.

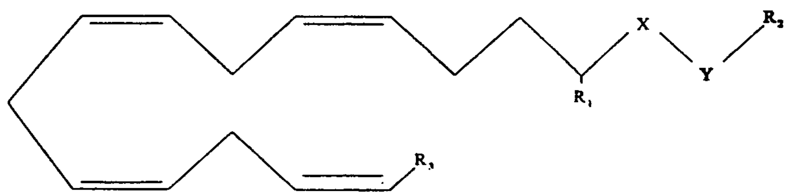
Retro-anandamides are defined as anandamide analogs in which the position of the NH and CO groups have been reversed. Analogs 10, 11 and 12 are examples of some retro-anandamides. It should be noted that the retro-anandamides as a group show excellent affinity to, and selectivity for, the CB1 receptor. Further, the retro-anandamides show virtually no difference in CB1 affinities when tested with and without PMSF. Thus the retro-anandamides exhibit excellent metabolic stability. The results demonstrate that the retro-anandamides are not substrates for anandamide amidase and therefore are not susceptible to its hydrolytic actions.

PROPHETIC EXAMPLE 2

Based on the above testing and results, it is believed that lipid compounds based on structural formulas 1 and 2, illustrated in Table 2, would exhibit increased cannabinoid receptor affinities and/or selectivities as well as increased metabolic stability. In fact, structural formulas 1 and 2 include analogs 1 - 12 discussed above.

TABLE 2

Structural Formula 1:



X is one of the group consisting of C=O and NH and Y is the other of that group. Expressed another way, X may be C=O and Y may be NH, or Y may be C=O and X may be NH, but both X and Y may not be the same group.

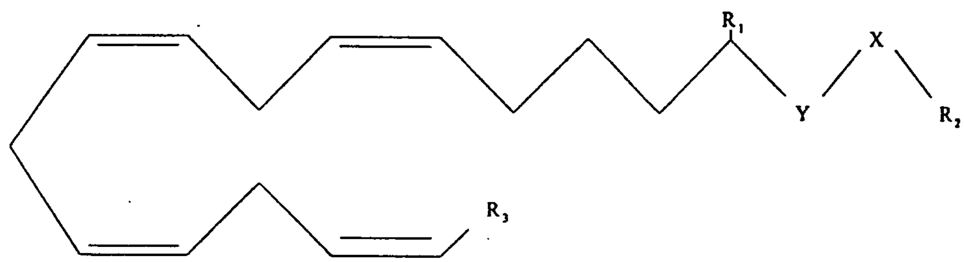
R_1 is selected from the group consisting of H and alkyl groups. More specifically, R_1 is selected from the group consisting of H, CH_3 and $(CH_3)_2$.

R_2 is selected from the group consisting of alkyl, substituted alkyl, alkenyl and alkynyl groups. More specifically, R_2 is selected from the group consisting of $CH(R)CH_2Z$, $CH_2CH(R)Z$ and $CH(R)(CH_2)nCH_2Z$, R being selected from the group consisting of H, CH, CH_3, CHCH, CH_2CF_3 and $(CH_3)_2$, Z being selected from the group consisting of H, halogens, N_3, NCS and OH and n being selected from the group consisting of 0, 1 and 2.

R_3 is selected from the group consisting of alkyl, substituted alkyl, aryl, alkylaryl, O-alkyl, O-alkylaryl, cyclic and heterocyclic groups. As used herein, O-alkyl and O-alkylaryl refer to groups in which an oxygen atom is interposed between carbon atoms on the anandamide portion and substituent group. Non-limiting examples of such R_3 groups include cyclohexyl, cyclopentyl, alkylcyclohexyl, alkylcyclopentyl, piperidinyl,

morpholinyl and pyridinyl. More specifically, R_3 is selected from the group consisting of n-$C_5H_{10}Z'$, n-$C_6H_{12}Z'$, n-$C_7H_{14}Z'$ and 1',1'-$C(CH_3)_2(CH_2)_5CH_2Z'$, Z' being selected from the group consisting of H, halogens, CN, N_3, NCS and OH.

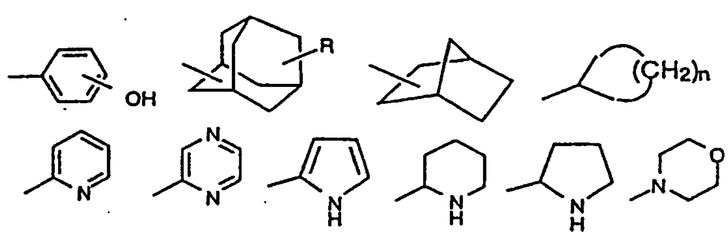
Structural Formula 2:



Y is one of the group consisting of C=O and NH and X is the other of that group.

R_1 is selected from the group consisting of H and alkyl groups. More specifically, R_1 is selected from the group consisting of H, CH_3 and $(CH_3)_2$.

R_2 is selected from the group consisting of alkyl, substituted alkyl, alkenyl, alkynyl, O-alkyl, cyclic, polycyclic and heterocyclic groups. More specifically, R_2 is selected from the group consisting of



$CH{=}CH_2$, $CH{=}C(CH_3)_2$, $C{\equiv}CH$, CH_2OCH_3, $CH(R)(CH_2)nCH_2Z$ and $CH_2CH(R)(CH_2)nZ$, R being selected from the group consisting of H, CH_3 and $(CH_3)_2$, Z being selected from the group consisting of H, halogens,

N_3, NCS, OH and OAc and n being selected from the group consisting of 0, 1 and 2; and

R_3 is selected from the group consisting of alkyl, substituted alkyl, aryl, alkylaryl, O-alkyl, O-alkylaryl, cyclic and heterocyclic groups. Non-limiting examples of such R_3 groups include cyclohexyl, cyclopentyl, alkylcyclohexyl, alkylcyclopentyl, piperidinyl, morpholinyl and pyridinyl. More specifically, R_3 may be selected from the group consisting of n-$C_5H_{10}Z'$, n-$C_6H_{12}Z'$, n-$C_7H_{14}Z'$ and 1',1'-$C(CH_3)_2(CH_2)_5CH_2Z'$, Z' being selected from the group consisting of H, halogens, CN, N_3, NCS and OH.

Those skilled in the art will recognize, or be able to ascertain with no more than routine experimentation, many equivalents to the specific embodiments of the invention disclosed herein. Such equivalents are intended to be encompassed by the scope of the invention.